

# Effect of tracheal mucus and tracheal cytology on racing performance in Thoroughbred racehorses

S. J. HOLCOMBE\*, N. E. ROBINSON, F. J. DERKSEN, B. BERTOLD†, R. GENOVESE†, R. MILLER, H. DE FEITER RUPP, E. A. CARR, S. W. EBERHART, D. BORUTA and J. B. KANEENE

*Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan 48824; †Randall Veterinary Hospital, Inc. and Bertold Equine Veterinary Services, 20600 Miles Parkway, Warrensville Heights, Ohio 44128-5504, USA.*

**Keywords:** horse; mucus; neutrophil; trachea; nasopharynx

## Summary

**Reason for performing study:** Accumulations of mucus within the trachea are often found during endoscopic examinations of the airways of poorly performing racehorses, but the clinical importance of this finding is unknown.

**Objectives:** To determine the effect of tracheal mucus, pharyngeal lymphoid hyperplasia (PLH) and cytological indices of tracheal aspirate on racing performance in Thoroughbred horses assessed by race place and whether the horse was raced.

**Methods:** Endoscopic examination of the nasopharynx, larynx and trachea was performed, and a tracheal aspirate obtained monthly at Thistledown racetrack from April to December, 2002 and 2003. Horses received a score of 0–4 for the degree of PLH and 0–4 for the amount of mucus visible in the trachea. The tracheal aspirate was assessed for turbidity, and total and differential cell counts. Generalised estimating equations models were used as repeated measures models for each risk factor and the level of association assessed through the risk factor's P value in the model.

**Results:** Moderate to severe tracheal mucus (2–4) was a risk factor for poor racing performance. There was no association between degree of PLH, cell counts or turbidity of tracheal wash fluid and racing performance. However, horses that raced had higher total neutrophil counts in tracheal wash aspirates than horses that did not race.

**Conclusions:** Grades 2–4 tracheal mucus should be considered a potential cause of poor racing performance in Thoroughbred horses.

**Clinical relevance:** Because moderate to severe tracheal mucus accumulation, and not increased tracheal neutrophils, was a risk factor for poor racing performance, functionally significant airway inflammation may best be confirmed by the presence of mucus rather than increased number of neutrophils in the trachea.

## Introduction

Epidemiological studies of the lower respiratory tract have demonstrated that although clinical signs of tracheobronchial inflammation such as coughing are associated with the isolation of

pathogenic bacteria, accumulations of mucus and inflammatory cells are frequently present in the trachea in the absence of clinical signs, such as cough and malaise (Sweeney *et al.* 1992; Wood *et al.* 1993; Burrell *et al.* 1996; Chapman *et al.* 2000; Christley *et al.* 2001a,b).

Although there are data to suggest that airway mucoid accumulations may cause ventilation perfusion mismatching and impaired gas exchange in horses (Couetil and Denicola 1999; Sanchez *et al.* 2005), the effect of lower airway inflammation on performance has not been reported in a population of Thoroughbred racehorses. Racing is the ultimate lung function test because, in order to supply the maximal oxygen consumption necessary to win a race, horses need healthy airways and lungs. Horses with airway inflammation are therefore likely to have reduced racing performance. This was confirmed by MacNamara *et al.* (1990), who found that Standardbreds with accumulations of tracheal mucus were more likely to finish last or next to last rather than first or second in a race. This study, however, did not control for variables that also affect racing performance, such as age, gender and trainer.

In addition to lower airway inflammation, young horses commonly develop inflammation of the nasopharynx known as pharyngeal lymphoid hyperplasia (PLH). The location of the nasopharynx at the entrance of the airway exposes it to multiple types of allergens, irritant particles and viral or bacterial agents. The local lymphoid tissue responds by stimulating mucus-producing cells and by producing local immunoglobulins. As young horses start their performance careers, enter training barns and travel, they become exposed to multiple new antigenic stimuli. Therefore, PLH is very common in young horses but has not been associated with diminished racing performance (Auer *et al.* 1985; Hobo *et al.* 1995). However, there are anecdotal concerns that pharyngitis may be a prelude to dynamic upper airway obstruction and the sequelae of nasopharyngeal inflammation may be more performance limiting than the initial bout of pharyngitis. Accumulating evidence suggests that regional inflammation of the upper airway may predispose individuals to obstructive upper airway disease, such as nasopharyngeal collapse, dorsal displacement of the soft palate and aryepiglottic fold collapse (King *et al.* 2001). Therefore, the purpose of this study was to determine if tracheal mucus accumulation and large airway inflammation are associated with poor racing performance in Thoroughbred horses.

\*Author to whom correspondence should be addressed.

[Paper received for publication 04.10.05; Accepted 15.02.06]

## Materials and methods

### Horses

Participation of owners and trainers and their horses was solicited at Thistledown Racetrack in Cleveland, Ohio from April of 2002 to October of 2003. The All-University Committee for Animal Use and Care at Michigan State University approved the protocol. All horses used in the study were housed principally at the racetrack and under the care of their trainers, attendants and veterinarians. No aspect of the horses' daily routine, feeding, medication and exercise schedule was altered because of enrolment in the study.

### Experimental design

Endoscopic examination of the nasopharynx, larynx and trachea was performed and a tracheal aspirate obtained from horses, monthly, for a maximum of 9 examinations. All examinations and sample collections were performed by the same veterinarian (S.J.H), a second veterinarian and a licensed veterinary technician. Endoscopic examinations were performed in resting horses, mid-morning to afternoon, with a minimum time between racing and examination of 24 h.

**Methods of evaluation:** Endoscopic examinations were performed with the horse in its own stall, restrained with a lip chain by a trainer or groom from the racing stable. The endoscope was passed through the right nostril and advanced along the ventral meatus, caudally to the nasopharynx. The nasopharynx and larynx were examined and the degree of pharyngeal lymphoid hyperplasia determined (Raker and Boles 1978). The occurrence of dorsal displacement of the soft palate, nasopharyngeal collapse, or structural or functional laryngeal abnormalities was noted. The endoscope was advanced caudally into the trachea to the level of the carina. At that time, the amount of mucus in the trachea was graded (Gerber *et al.* 2004a). The endoscope was then retracted to the cranial third of the trachea, a sterile polytetrafluoroethylene catheter<sup>1</sup> passed through the biopsy channel of the endoscope into the trachea and 10 ml saline infused through the catheter into the trachea. The endoscope was advanced caudally into the trachea until the saline pool was seen and then aspirated. The endoscope was cleaned with betadine solution and rinsed with distilled water between horses.

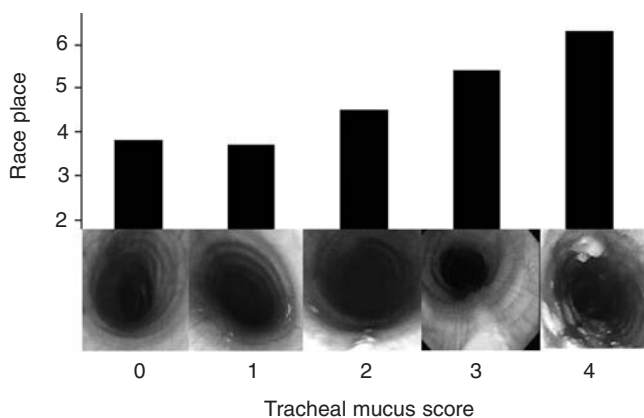


Fig 1: Graph of race place based on tracheal mucus score. The average race place increases as the tracheal mucus score increases above 1. Tracheal mucus images from Gerber *et al.* (2004a).

**Scoring large airway inflammation and function:** Horses received a score of 0–4 for pharyngeal lymphoid hyperplasia (PLH) (Raker *et al.* 1978). Briefly, 0, if there was no evidence of follicular hyperplasia; 1, if hyperplasia was <180° of the tonsillar margin; 2, if small follicles were present along the full circumference of the tonsillar margin with mild oedema of the dorsal pharyngeal recess; 3, if moderate follicular hyperplasia was noted circumferentially with moderate oedema and hyperplasia of the dorsal pharyngeal recess; and 4, if severe follicular hyperplasia with coalescing follicles and severe oedema and follicular hyperplasia of the dorsal pharyngeal recess was seen.

**Scoring tracheal mucus:** The trachea was examined from the most proximal region to the carina. Mucus was scored (Fig 1) based on the scoring system verified by Gerber *et al.* (2004a). Briefly: 0 no mucus; 1, small, singular threads or droplets of mucus; 2, larger, confluent droplets of mucus; 3, streams of mucus within the trachea; and 4, large streams or pools of mucus that covered greater than 25% of the tracheal circumference.

**Tracheal lavage:** Tracheal aspirates were transferred to 15 ml nonpyrogenic polypropylene tubes<sup>2</sup> and stored on ice for approximately 30 min until samples from 12 h were obtained. At that time, the samples were transported to a field laboratory where total cells were counted and slides prepared for differential cell counts. Lavage samples were graded 1, 2 or 3, based on the turbidity of the solution.

Tracheal wash samples graded 2 or 3 for turbidity were diluted (125 µl tracheal wash and 375 µl sterile buffer). In the case of extremely viscous samples, further dilutions were made as necessary. For each sample, 2 slides were prepared by use of a cytocentrifuge. Each slide was loaded with 175 µl of sample and the positively charged slides spun at 600 rpm for 8 mins, removed from the cassette, air dried and sprayed with histological fixative for transport to Michigan State University. For total cell count, 10 µl of diluted sample was loaded into one side of a haemocytometer. White blood cells were counted in 4 chambers and total cell count was determined from the following formula: cell concentration/ml = total cell count in 4 squares x 2500 x dilution factor.

Differential cell counts were performed at Michigan State University on the cytospin slides stained with Wright-Giemsa examined under a microscope using a 40x objective. A total of 200 leucocytes were counted in order to determine the percentage of neutrophils, macrophages, lymphocytes, eosinophils and mast cells. Absolute cell counts were calculated from the total and differential cell counts.

**Performance:** Race records for each horse were obtained from the Jockey Club. Racing records within 2 weeks of sampling were examined and race place was recorded for the race closest to the time of sampling, within the 2 week period. If the horse did not race within this time window, it was recorded as not racing for that examination period.

### Statistical analysis

For simple descriptive statistics, Fisher's Exact 2-tailed test was used to describe associations between PLH and mucus scores. For continuous risk factors (animal age, tracheal cytology), analysis of variance (ANOVA) was used to describe associations between these factors and inflammation scores (PLH and mucus).

Since multiple race outcomes for each horse were collected, repeated measures modelling approaches were used. Generalised estimating equations (GEE) models were used as repeated measures models for each risk factor for race place and the level of association was assessed through the risk factor's P value in the model. Two series of GEE models were developed for different individual horse performance outcomes. Logistic regression models were developed for racing (yes/no) and linear regression models were developed for race place. GEE models were constructed for each individual airway inflammation risk factor, controlling for the effect of trainer (total of 13 trainers, 7 in year 1, 6 new trainers in year 2 with 4 trainers returning from year one), time between sample collection and racing (days), race value (dollars) and horse age (years), which were considered to be extrinsic factors affecting race performance. The effect of risk factors were reported as odds ratios based on empirical standard error estimates of parameters:  $OR = e^{\beta}$  where  $\beta$  = parameter estimate for the given risk factor from the model.

Multivariable models were generated using a hierarchical backwards model-building procedure. Risk factors with  $P \leq 0.20$  in univariable analysis were considered for inclusion in the multivariable model. Interaction terms were developed based on biological plausibility and the results of descriptive statistics. Risk factors selected for elimination by removal of the risk factor with the highest P value. The reduced model was executed and confounding assessed by computing odds ratios for risk factors that remained in the model. If odds ratios of any of the remaining risk factors experienced changes  $>10\%$ , the removed variable was considered to be a confounder for the remaining risk factors and

was retained in the model. The model with the best combination of lowest model deviance and covariate P values ( $P \leq 0.05$ ) was considered to be the final reduced form of the multi variable model.

**Results**

One to 7 examinations were performed on 525 horses during the study. Race records were obtained for 455 horses and 327 were used in the analysis assessing racehorse performance: 128 horses with race records were eliminated from the analysis for various reasons, including lameness, colic or incomplete endoscopic examination or tracheal wash data. Displaced soft palate was reported in 5.2% (99 of 891) examinations. There were 169 mares, 49 colts and 114 geldings. Horses had a median age of 3 years (mean 3.74 years). Of 1,027 completed races, the mean race value was \$14,020 (95% C.I. = \$13,190 - \$14,850). There was a significant negative association between age and race value in \$1000s (odds ratio = 0.38, 95% C.I. = 0.16 - 0.88), controlling for trainer, which suggests that older horses were more likely to race, but in lower quality races.

*Associations between PLH, mucus and age*

Pharyngeal lymphoid hyperplasia (PLH) was significantly associated with age: horses with more severe PLH were significantly younger than horses with lower PLH scores (Table 1). Tracheal mucus was also associated with age (Table 2), with the average age of horses decreasing as mucus scores increased. There was a statistically significant positive association between age and

**TABLE 1: Associations between animal age and grade of pharyngeal lymphoid hyperplasia (PLH)**

Grade of PLH	Age (years)			ANOVA	
	n	Mean	s.e.	F	P
0	237	5.5	0.1	265.52	<0.0001
1	744	4.1	0.1		
2	692	2.9	0.04		
3	197	2.4	0.1		
4	28	2.0	0.04		

**TABLE 2: Associations between animal age and grade of tracheal mucus**

Grade of mucus	Age (years)			ANOVA	
	n	Mean	s.e.	F	P
0	1116	3.9	0.1	29.24	<0.0001
1	529	3.4	0.1		
2	140	3.1	0.1		
3	85	2.7	0.1		
4	30	2.4	0.1		

**TABLE 3: Associations between mucus score and grade of pharyngeal lymphoid hyperplasia (PLH)**

Grade of PLH	Mucus score						Fisher's exact 2-tailed P
	0	1	2	3	4	5	
0	174	50	9	4	0	0	<0.0001
1	485	193	42	18	6	0	
2	360	214	63	44	10	1	
3	86	63	20	18	10	0	
4	10	9	6	0	3	0	

**TABLE 4: Tracheal wash cytology (cells/ $\mu$ ) by pharyngeal lymphoid hyperplasia (PLH) scores**

Index	PLH Score (samples)					ANOVA	
	0	1	2	3	4	F	P
Total cells	3.9	4.2	5.7	6.9	5.1	1.8	0.1200
Total neutrophils	1.6	2.3	3.4	4.4	1.6	1.6	0.1652
Total macrophages	1.8	1.6	1.9	2.3	3.0	2.3	0.0529
Total lymphocytes	0.4	0.3	0.4	0.4	0.5	1.0	0.4101
Total eosinophils	0.01	0.02	0.01	0.01	0.01	0.9	0.4817
Total mast cells	0.01	0.01	0.01	0.01	0.02	0.6	0.6336

**TABLE 5: Tracheal wash cytology (cells/ $\mu$ ) by mucus scores**

Risk factor	Mucus score					ANOVA	
	0	1	2	3	4	F	P
Total cells	3.0	4.2	7.5	20.5	37.0	64.1	<0.0001
Total neutrophils	1.3	1.9	4.2	14.8	30.4	55.3	<0.0001
Total macrophages	1.3	1.9	2.6	4.4	5.7	26.3	<0.0001
Total lymphocytes	0.3	0.4	0.5	0.9	0.9	17.9	<0.0001
Total eosinophils	0.01	0.02	0.04	0.01	0.02	4.8	0.0007
Total mast cells	0.01	0.01	0.01	0.02	0.05	5.9	<0.0001

**TABLE 6: Multivariable generalised equalising equations (GEE) repeated measures regression models for racing (yes/no) by PLH and mucus scores, controlling for year, trainer, time from sampling to racing, and horse age and gender, measures repeated within horse, 2002–2003**

Risk factor	Model log likelihood	P	Odds ratio	95% C.I.
PLH (0–4)	1277.04	<0.0001	0.92	0.89–0.95
Mucus (0–5)	1286.66	0.0002	0.95	0.93–0.98
Mucus (low/high)	1289.14	0.0021	0.90	0.84–0.96

finishing a race (odds ratio = 1.07, 95% C.I. = 1.05 - 1.10), but no associations were found between age and race place.

#### *Associations between PLH, mucus and tracheal wash cytology*

The degree of PLH also was associated with tracheal mucus score, such that as degree of PLH increased, tracheal mucus score increased (Table 3). In general, there were few significant associations found between PLH scores and tracheal wash cytology. Significant associations were found between PLH scores and total number of macrophages, with the number of cells increasing as PLH scores increased (Table 4). Significantly higher numbers of total cells, neutrophils, macrophages and lymphocytes associated with increasing mucus scores (Table 5).

#### *Effects on performance: racing (yes/no)*

Based on the results of repeated measures multivariable regression models, increasing PLH score was associated with decreased likelihood of racing (Table 6). Similarly, increasing mucus scores were associated with decreased likelihood of racing. In the multivariable model, there were significant associations between tracheal wash cytology and race completion (Table 7).

#### *Effects on performance: race place*

The degree of PLH was not significantly associated with race place (Table 8), but mucus, when categorised as low (0–1) or high (2–4), was associated with race place: race place increased with high mucus scores (Table 8, Fig 1). There was no significant association between total cells, total neutrophils, total macrophages, total lymphocytes, total eosinophils, or percent cell types and race place (Table 9). Attempts to generate a multivariable model for tracheal cytology scores did not result in models meeting criteria for significance.

## Discussion

The results of this study suggest that moderate to severe tracheal mucus accumulation is a risk factor for poor racing performance in Thoroughbred horses. These results seem intuitive if tracheal mucus is a reflection of mucus within the peripheral airways of the lungs, because mucus can obstruct airways and limit lung function. Adding credence to this postulation, the results of studies investigating the effect of neutrophilic inflammation in the bronchoalveolar lavage fluid and increased amounts of tracheal mucus on physiological measurements of gas exchange during exercise showed that affected horses had significantly lower arterial PaO<sub>2</sub>, higher heart rates and blood lactate concentrations compared to controls during incremental treadmill exercise (Couetil and Denicola 1999; Sanchez *et al.* 2005). These results, combined with the results of the present study, suggest that lung ventilation perfusion inequality may be of sufficient magnitude in horses with excess airway mucus to limit lung function and oxygen delivery, ultimately leading to poor racing performance. It will be important, however, to show that tracheal mucus reflects the amount of mucus present in the lungs.

Production of mucus can be initiated by neutrophilic inflammation or directly by irritants including bacteria, viruses and environmental contaminants such as endotoxin, hay dust and pollutants (Robinson 2001, 2003; Gerber *et al.* 2004a). In the present

study, there was a positive association between increasing numbers of neutrophils in the tracheal aspirate and increasing amounts of tracheal mucus. However, despite the fact that moderate to severe amounts of tracheal mucus were associated with decreased racing performance, no such association was found for any cytological indices of the tracheal aspirate, including increasing numbers of tracheal neutrophils. In fact, increased numbers of tracheal neutrophils were associated with an increased likelihood of racing in this study when the tracheal aspirate cytology results from horses that raced within 2 weeks of examination were compared to those horses that did not race. This leads to the conclusion that neutrophilic inflammation with increased mucus production was associated with decreased racing performance but high levels of neutrophils in the trachea, in the absence of mucus, was not a risk factor and was actually associated with an increased likelihood of racing. This dichotomy may be explained in 2 ways; firstly, an increased number of tracheal neutrophils may be an adaptation to increased exercise and not a pathogenic response (Bonsignore *et al.* 2003) secondly, the increased number may not correlate with an increased neutrophil count within the lung, as demonstrated in tracheal aspirates and bronchoalveolar lavage cytology (Derksen *et al.* 1989; Hewson *et al.* 2005). Previously, McCane *et al.* (1993) had found that bronchoalveolar lavage fluid from horses in active training or racing contained approximately twice as many neutrophils as those undertaking slow work. Similar results have been found in human athletes (Bonsignore *et al.* 2001, 2003).

**TABLE 7: Multivariable generalised equalising equations (GEE) repeated measures regression model for racing (yes/no) by tracheal wash cytology (cells/ $\mu$ l), controlling for year, trainer, race value, time from sampling to racing, and horse age and gender, measures repeated within horse, 2002–2003**

Risk factor	Coefficient	s.e.	P	Odds Ratio	95% C.I.
Neutrophils	0.2173	0.0839	0.0095	1.24	1.05–1.47
Macrophages	0.2122	0.0834	0.0110	1.24	1.05–1.46
Lymphocytes	0.2485	0.0954	0.0092	1.28	1.06–1.55

Model log likelihood = -1111.56

**TABLE 8: Multivariable generalised equalising equations (GEE) repeated measures regression models for race place by PLH and mucus scores, controlling for year, trainer, time from sampling to racing, and horse age and gender, measures repeated within horse, 2002–2003**

Risk factor	Model log likelihood	P	Odds ratio	95% C.I.
PLH (0-4)	2369.92	0.1234	1.20	0.95–1.52
Mucus (low/high)	2372.16	0.0347	1.72	1.04–2.84

Mucus scores: low = 0–1 and high = 2–4

**TABLE 9: Multivariable generalised equalising equations (GEE) repeated measures regression models for race place by tracheal wash cytology (cells/ $\mu$ l), controlling for year, trainer, race value, time from sampling to racing, and horse age and gender, measures repeated within horse, 2002–2003**

Risk factor	Model log likelihood	P	Odds ratio	95% C.I.
Total cell count	-2078.78	0.7459	1.00	0.89–1.03
Total neutrophils	-2056.49	0.7900	1.00	0.97–1.04
Total macrophages	-2056.52	0.7433	1.008	0.96–1.05
Total lymphocytes	-2056.23	0.2996	0.88	0.70–1.12
Total eosinophils	-2054.13	0.2886	1.85	0.59–5.78
Total mast cells	-2056.55	0.9598	1.12	0.02–86.04

Pharyngeal lymphoid hyperplasia (PLH) was not identified as a risk factor for poor racing performance in this study. We hypothesised that such an association might be found because inflammation of the upper airway has been associated with obstructive upper airway diseases such as dorsal displacement of the soft palate, aryepiglottic fold collapse nasopharyngeal collapse and epiglottic entrapment (King *et al.* 2001). Some horses affected with these diseases respond favourably to rest and anti-inflammatory therapy, suggesting that localised inflammation or PLH might play a role in the aetiopathogenesis of these diseases (King *et al.* 2001). This may be because many horses, especially young horses, develop grades 2–4 PLH but only a small subset suffer dynamic nasopharyngeal or laryngeal collapse as sequelae. The present study found younger horses had more severe PLH than older horses, and other studies have found a similar association between age and degree of PLH (Auer *et al.* 1985; Hobo *et al.* 1995). Because the results of our study and others have not found PLH to be a risk factor for poor racing performance, PLH alone should not be a reason to prevent a horse from being raced (Auer *et al.* 1985; Hobo *et al.* 1995).

In conclusion, moderate to severe amounts of tracheal mucus were associated with decreased racing performance in Thoroughbred horses. In agreement with other investigations, PLH was not associated with poor performance, but was associated with age, such that younger horses were more prone to increased grades of PLH. Tracheal mucus score was positively associated with increased inflammatory indices in tracheal aspirate fluid, but increased tracheal neutrophil count was not associated with decreased racing performance. Therefore, functionally significant airway inflammation may best be confirmed by the presence of tracheal mucus rather than increased numbers of neutrophils in the tracheal aspirate. It is emphasised that horses in this study were considered healthy enough to race, and that findings should be extrapolated only to similar equine populations.

## Acknowledgement

Funded by the Grayson-Jockey Club Research Foundation.

## Manufacturers' addresses

<sup>1</sup>Cole-Palmer Instruments, Vernon Hills, Illinois, USA.

<sup>2</sup>Corning Inc., Corning, New York, USA.

## References

- Auer, D.E., Wilson, R.G. and Groenendyk, S. (1985) Pharyngeal lymphoid hyperplasia in Thoroughbred racehorses in training. *Aust. vet. J.* **62**, 124–6.
- Bonsignore, M.R., Morici, G., Riccobono, L., Insalaco, G., Bonanno, A., Profita, M., Paterno, A., Vassalle, C., Mirabella, A. and Vignola, A.M. (2001) Airway inflammation in nonasthmatic amateur runners. *Am. J. Physiol. Lung. Cell. Mol. Physiol.* **281**: L668–L676.
- Bonsignore, M.R., Morici, G., Vignola, A.M., Riccobono, L., Bonanno, A., Profita, M., Abate, P., Scichilone, N., Amato, G., Bellia, V. and Bonsignore, G. (2003) Increased airway inflammatory cells in endurance athletes: what do they mean? *Clin. exp. Allergy* **33**, 14–21.
- Burrell, M.H., Wood, J.L.N., Whitwell, K.E., Chanter, N., Mackintosh, M.E. and Mumford, J.A. (1996) Respiratory disease in thoroughbred horses in training: the relationships between disease and viruses, bacteria and environment. *Vet. Rec.* **139**, 308–313.
- Chapman, P.S., Green, C., Main, J.P., Taylor, P.M., Cunningham, F.M., Cook, A.J. and Marr, C.M. (2000) Retrospective study of the relationships between age, inflammation and the isolation of bacteria from the lower respiratory tract of thoroughbred horses. *Vet. Rec.* **146**, 91–95.
- Christley, R.M., Hodgson, D.R., Rose, R.J., Hodgson, J.L., Wood, J.L. and Reid, S.W. (2001a) Coughing in thoroughbred racehorses: risk factors and tracheal endoscopic and cytological findings. *Vet. Rec.* **148**, 99–104.
- Christley, R.M., Hodgson, D.R., Rose, R.J., Wood, J.L., Reids, S.W., Whitear, K.G. and Hodgson, J.L. (2001b) A case-control study of respiratory disease in Thoroughbred racehorses in Sydney, Australia. *Equine vet. J.* **33**, 256–264.
- Couetil, L.L. and Denicola, D.B. (1999) Blood gas, plasma lactate and bronchoalveolar lavage cytology analyses in racehorses with respiratory disease. *Equine vet. J., Suppl.* **30**, 77–82.
- Derksen, F.J., Brown, C.M., Sonea, I., Darien, B.J. and Robinson, N.E. (1989) Comparison of transtracheal aspirate and bronchoalveolar lavage cytology in 50 horses with chronic lung disease. *Equine vet. J.* **21**, 23–26.
- Gerber, V., Straub, R., Marti, E., Hauptman, J., Herholz, C., King, M., Tahon, L. and Robinson, N.E. (2004a) Endoscopic scoring of mucus quantity and quality: observer and horse variance and relation to mucus viscosity and airway inflammation. *Equine vet. J.* **36**, 576–82.
- Gerber, V., Lindberg, A., Berney, C. and Robinson, N.E. (2004b) Airway mucus in recurrent airway obstruction—short-term response to environmental challenge. *J. vet. int. Med.* **18**, 92–97.
- Hewson, J. and Viel, L. 2005, *Streptococcus* in the trachea: is it merely a contaminant? *Proceedings of the World Equine Airway Symposium*, Ithaca, New York pp 51–53.
- Hobo, S., Matsuda, Y. and Yoshida, K. (1995) Prevalence of upper respiratory tract disorders detected with a flexible videoendoscope in thoroughbred racehorses. *J. vet. med. Sci.* **57**, 409.
- King, D.S., Tulleners, E., Martin, B.B. Jr., Parente, E.J. and Boston, R. (2001) Clinical experiences with axial deviation of the aryepiglottic folds in 52 racehorses. *Vet. Surg.* **30**, 151–60.
- MacNamara, B., Bauer, S. and Iafe, J. (1990) Endoscopic evaluation of exercise-induced pulmonary hemorrhage and chronic obstructive pulmonary disease in association with poor performance in racing Standardbreds. *J. Am. vet. med. Ass.* **196**, 443–445.
- McKane, S.A., Canfield, P.J. and Rose, R.J. (1993). Equine bronchoalveolar lavage cytology: survey of Thoroughbred racehorses in training. *Aust. vet. J.* **70**, 401–404.
- Raker, C.W. and Boles, C.L. (1978) Pharyngeal lymphoid hyperplasia in the horse. *J. equine med. Surg.* **2**, 202–207.
- Sweeney, C.R., Humber, K.A. and Roby, K.A.W. (1992) Cytologic findings of tracheobronchial aspirates from 66 Thoroughbred racehorses. *Am. J. vet. Res.* **53**, 1172–1175.
- Robinson, N.E. (2001) Chairperson's introduction: International Workshop on Equine Chronic Airway Disease, Michigan State University, 2000. *Equine vet. J.* **33**, 5–19.
- Robinson, N.E. (2003) Workshop report: Inflammatory airway disease: defining the syndrome. *Equine vet. Educ.* **5**, 81–82.
- Sanchez, A., Couetil, L.L., Ward, M.P. and Clark, S.P. (2005). Effect of airway disease on blood gas exchange in racehorses. *J. vet. int. Med.* **19**, 87–92.
- Wood, J.L., Burrell, M.H., Roberts, C.A., Chanter, N. and Shaw, Y. (1993) *Streptococci* and *Pasteurella* spp. associated with disease of the equine lower respiratory tract. *Equine vet. J.* **25**, 314–318.

**Author contributions** S.J.C. and N.E.R. initiated, conceived and planned this study, and were assisted in its execution and data collection by F.J.D., B.B., R.G., H.d.F.R., E.A.C. S.W.E. and D.B. The article was written by S.J.C., N.E.R., F.J.D. and J.B.K.; statistics were by R.M. and J.B.K.